M E M O R A N D U M

Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation Research
Office of Blood Research and Review

To:	BLA STN 125506/0 and Pratibha Rana, OBRR/DBA/RPMB
From:	Andrey Sarafanov, PhD, OBRR/DH/LH
Applicant:	Bio Products Laboratory
Product:	Coagulation Factor X (Human)
Subject:	Chemistry, Manufacturing and Controls Review (validation of analytical methods)
Through:	Mark Weinstein, PhD, OBRR/IOD
	Basil Golding, MD, Director, DH/OBRR
CC:	Tim Lee, PhD and Mikhail Ovanesov, PhD; OBRR/DH/LH

EXECUTIVE SUMMARY

This memorandum summarizes a review of the analytical methodology used for testing the final drug product (DP) in an original Biologics License Application (BLA) under STN 125505, submitted by Bio Products Laboratory (BPL). The proposed DP is Coagulation Factor X (Human). Sections 3.2.P.5.2 and 3.2.P.5.3 (analytical procedures description and validation) and 3.2.P.6 (reference materials) were reviewed. We found that a number of methods were not validated or not validated sufficiently. For most issues, informational requests (IR) were sent by the Division of Biological Standards and Quality Control (DBSQC). I sent an additional IR request on November 22, 2013, and received satisfactory responses from BPL, on December 5, 2013 (Amendment 19) and on January 31, 2014 (Amendment 26). Nevertheless, by the review due date (February 21, 2014), major issues had not been sufficiently resolved, including those in a review memo from Dr. Lokesh Bhattacharyya (DBSQC), and thus approval is not recommended at this time.

BACKGROUND

Factor X (FX) is a sterile, freeze-dried concentrate of human coagulation factor X. It is produced from human plasma, which is collected at FDA licensed centers in the US. The manufacturing process of FX includes three dedicated virus inactivation steps: solvent-detergent (S/D) treatment, virus-filtration, and terminal dry heat treatment. The process does not have a defined (isolated) drug substance. There is no pharmacopeial monograph for FX concentrate; therefore, where appropriate, BPL adopted the requirements or intent of monographs used for the testing of other coagulation factor concentrates to test FX DP. The drug product (DP) is supplied as a freeze-dried powder, along with sterilized water for injection (WFI), and is presented in two dose sizes of 250 International Units (IU) and 500 IU (nominal). The 250 IU dose is reconstituted with the supplied 2.5mL WFI, and the 500 IU dose with 5mL WFI. After reconstitution, each dose size contains the same concentration of active ingredient and formulation chemicals, and differs only by their volumes. After reconstitution with WFI, FX is intended for use as a replacement therapy in patients with a hereditary deficiency of coagulation FX.

REVIEW SUMMARY

ANALYTICAL PROCEDURES (3.2.P.5.2 and 3.2.P.5.3)

Analytical procedures are used to ensure that the DP has qualities that match its specifications. The specifications are listed in Table 1 (3.2.P.5.1).

Table 1. **Drug Product Specifications.**

Test	Compliance	Test Limits
Characteristics		
Description of freeze-dried plug	BPL	Smooth white plug
Moisture (b) (4)	BPL	(b) (4)
Solubility at (b) (4)	BPL	
Appearance of solution	BPL	Colourless, clear or slightly opalescent solution.
(b) (4)		
Stability at (b) (4)	BPL	(b) (4)
Identity	BPL	Product complies with limits of factor X assay
Biological Safety Tests		
Sterility test	BPL	Pass
Bacterial Endotoxin Test (b) (4)	BPL	(b) (4)
General Safety Test	BPL	Pass
Purity/Specific Function		
Factor X activity, IU/mL	BPL	80 (b) (4)
Factor X per vial, IU/vial	BPL	200 (b) (4) (250 IU dose) 400 (500 IU dose)
(b) (4)		
Total Protein, g/L	BPL	(b) (4)
Specific activity, IU/mg protein	BPL	
NAPTT (b) (4)	BPL	
NAPTT (b) (4)	BPL	
FCT (b) (4)	BPL	
Excipients		
Chloride, (b) (4)	BPL	
Phosphate,	BPL	
Citrate	BPL	
Sucrose, (b) (4)	BPL	
Sodium, (b) (4)	BPL	
Impurities		
Factor II, IU/ml	BPL	NGT 1
Factor IX, IU/ml	BPL	NGT 1
(b) (4)		

NGT, Not Greater Than
NLT, Not Less Than
LT, Less Than

The relevant analytical methods are listed in the Table 2.

Table 2. **Analytical Procedures Used for Testing Factor X Drug Product.**

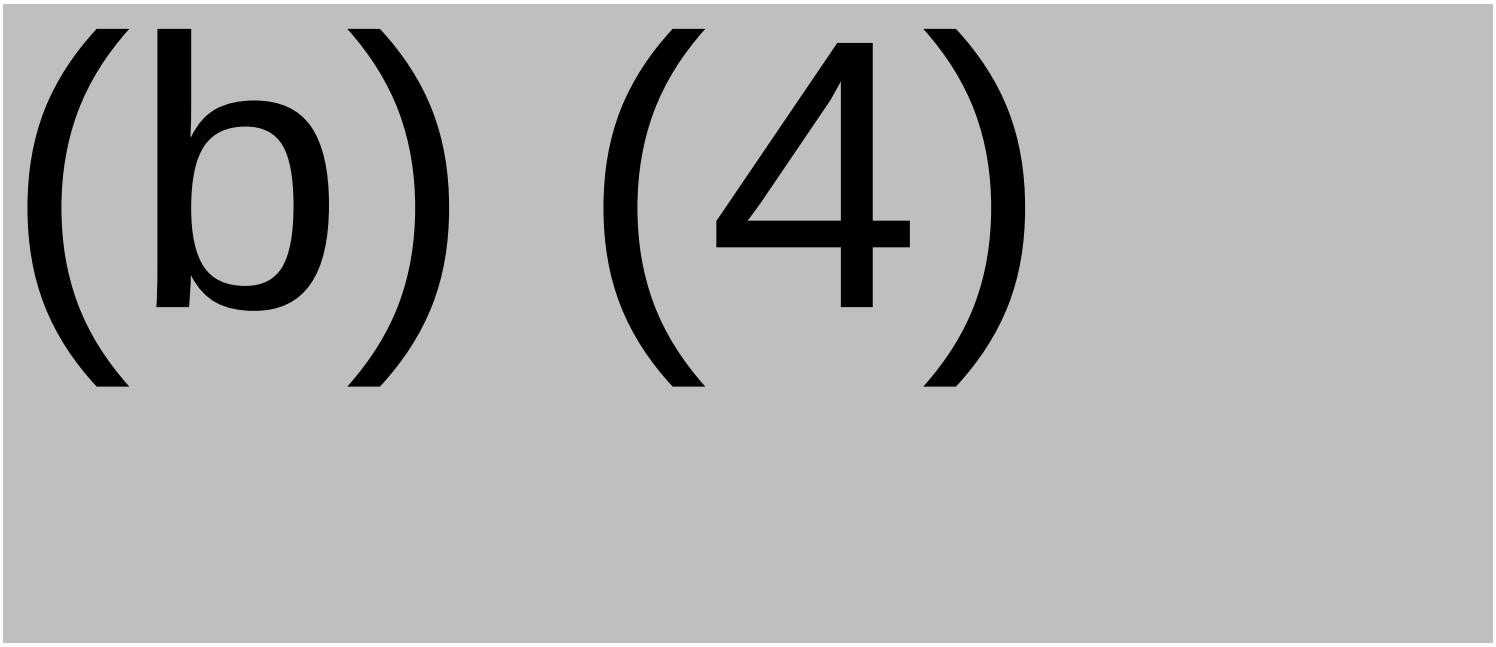
Analytical Test	Method	Compliance [a]	Pharmacopoeial principle
Characteristics			
Appearance of freeze-dried plug	Visual inspection	BPL	(b) (4)
Residual water content	(b) (4)	BPL	
Solubility	Visual inspection(b) (4)	BPL	
Appearance of solution	Visual inspection	BPL	
(b) (4)		BPL	
		BPL	
Stability	Visual inspection	BPL	
Identity	Assay	BPL	
Biological Safety Tests			
Sterility	Membrane filtration	BPL	
Endotoxin	(b) (4)	BPL	
General Safety Test	Animal test	BPL	CFR
Purity/Specific Function			
Factor X	Chromogenic	BPL	(b) (4)
Total Protein	(b) (4)	BPL	
Specific activity	Calculation	BPL	
NAPTT	Clotting time	BPL	
FCT	Clotting time	BPL	
Excipients			
Chloride	(b) (4)	BPL	
Phosphate		BPL	
Citrate		BPL	
Sucrose		BPL	
Sodium		BPL	
Impurities			
Factor II	(b) (4)	BPL	
Factor IX		BPL	
(b) (4)		BPL	
		BPL	
		BPL	

[a] There are no pharmacopeial monographs for human coagulation factor X. Compliance standards were set by BPL. The table indicates where the methods or limits were adopted from other pharmacopeial monographs.

Reviewer's Comment
Relevant sections of the monographs are not listed.

Validation parameters of the analytical methods are shown in Table 3.

Table 3. **Validation parameters of the analytical methods.**



Validation type: 1 - Quantitative assay; 2 - Quantitative impurity assay; 3 - Limit impurity assay; a - Fully validated using Factor X; b - Generic validation

Reviewer's Comment
Not all validated methods are listed in the Table 3. Parameters that are not listed are described under relevant sections of the submission.

3.2.P.5.2.1 Characteristics

3.2.P.5.2.1.1 Solubility, appearance of reconstituted solution, and stability at (b) (4)

The freeze-dried material is inspected, and WFI is then added to the material. Conformation is obtained for acceptable reconstitution time (b) (4)), appearance before and after reconstitution, and compliance with labeling criteria. As stated by the Applicant, no validation is necessary.

3.2.P.5.2.1.2 Determination of (b) (4)

The method is described in (b) (4) . Method validation (Precision) was performed using three batches of the DP. All validation criteria were met.

3.2.P.5.2.1.3 Determination of (b) (4)

The method is described in (b) (4) Method validation was performed using three batches of the DP. Specificity was considered not applicable. All validation criteria were met.

3.2.P.5.2.1.4 Determination of Moisture by the (b) (4)

The method is described in (b) (4) . Method validation (b) (4) to use of the assay for FX". Specificity was not examined as substances known to interfere in the (b) (4) assay are not present in the DP.

Reviewer's Comments

1. Validation of the method is not satisfactory, because the study did not use (b) (4) samples. A DBSQC reviewer had a similar concern and sent an IR to resolve the issue. By the time of the review completion, the issue had not been resolved.

2. It is unclear how the measured moisture parameter, expressed in (b) (4) is relevant to the specification parameter (b) (4) The data need to be expressed in the same units. FDA sent an IR to BPL to resolve the concern, and it was addressed appropriately.

3.2.P.5.2.1.5 Identity

The method is described in (b) (4). Identity is determined from the FX assay for functional activity and product compliance with the assay limits (3.2.P.5.2.3.1), and thus, no further validation is necessary.

3.2.P.5.2.2 Biological Safety Tests

3.2.P.5.2.2.1 Sterility

The test is described in (b) (4). Method validation was performed using (b) (4) DP from (b) (4) as described in (b) (4) (b) (4)

Reviewer's Comment
Although it would be more relevant if the test microorganisms had been (b) (4), the protocol that was implemented follows the (b) (4)

3.2.P.5.2.2.2 (b) (4) Endotoxin Test

The test is described in the (b) (4). Method validation was performed using three batches of the DP. The acceptance criteria for a valid test were: (b) (4) Data met the acceptance criteria.

3.2.P.5.2.2.3 General Safety Test

The general safety test was performed as described in 21 CFR §610.11 (animal test).

3.2.P.5.2.3 Purity/Specific Function

3.2.P.5.2.3.1 Determination of Factor X

The method follows the (b) (4) method for the assay of human coagulation Factor X. The activity of FX is determined by (b) (4) FX activity in the test sample is assigned by comparison with a FX standard preparation, which is tested by the same procedure. The standard is either an IS or a reference preparation, calibrated in IU. The Reference Standard is the current (b) (4) against the current WHO IS for FII and FX Concentrate. Method validation was performed using three batches of the DP, except that the Accuracy, Linearity and Range were tested using only standards. Specificity was not tested based on the (b) (4). statement that the method is specific to FX. The assay was considered suitable for the determination of FX.

Reviewer's Comment
Based on the literature (b) (4) , the assay is highly specific to FX. At the same time, for Accuracy, Linearity and Range, validation of the method is not satisfactory as should be performed using DP samples. A DBSQC reviewer had similar concern and sent an IR to resolve the issue. By the time of the review completion, the issue has not been resolved.

3.2.P.5.2.3.2 Determination of Total Protein

The test is described in the (b) (4) . The principle of the method is tha (b) (4) Reference Standard is (b) (4) Method validation was performed using three batches of the DP. Specificity was determined by assaying the FX with the excipients at their upper limits in the DP specification. The study used three batches of the DP, with exception of validation of Linearity and Range.

Reviewer's Comment
Linearity and Range validation should be done using the matrix of the DP, but not only standards. A DBSQC reviewer had similar concern and sent an IR to resolve the issue. By the time of the review completion, the issue has not been resolved.

3.2.P.5.2.3.3 Determination of Specific Activity

The purity of product and the level of contamination by other proteins are reflected by the ratio of functional activity (potency) of the active ingredient to the total mass of protein, i.e. the specific activity. Because the parameter is based on the calculation of results from two other assays, no validation was performed.

3.2.P.5.2.3.4 Determination of Non-Activated Partial Thromboplastin Time (NAPTT)

The test follows the (b) (4) method for the measurement of activated clotting factors. The method principle is that (b) (4) There is no reference standard for this test. The test is qualitative and specificity is deliberately broad in order to identify signs of activation in many potential stages in the clotting cascade. No validation of the method was performed, because the test is (b) (4)

Reviewer's Comment
The method is not compendial in the US, and thus needs to be validated. A DBSQC reviewer had similar concern and sent an IR to resolve the issue. By the time of the review completion, the issue has not been resolved.

3.2.P.5.2.3.5 Determination of Fibrinogen Clotting Time (FCT)

The test measures the time for thrombin to clot a known concentration of fibrinogen. The test is a modified version of the current (b) (4) test for thrombin. A reference standard is not applicable to this procedure. The negative control is the assay buffer. The BPL method uses (b) (4) The BPL positive control is (b) (4) . No validation of the method was performed, because the test is compendial (b) (4)

Reviewer's Comment
The method is not compendial in the US, and thus needs to be validated. A DBSQC reviewer had similar concern and sent an IR to resolve the issue. By the time of the review completion, the issue had not been resolved.

3.2.P.5.2.4 Excipients

3.2.P.5.2.4.1 Determination of Chloride

Chloride is determined (b) (4) Method validation was performed using three batches of the DP and (b) (4). Specificity was determined by performing the assay for chloride in the presence of other excipients at their upper specification limit. All validation criteria were met.

Reviewer's Comment
This is BPL method as that described under the (b) (4) is based on different principle.

3.2.P.5.2.4.2 Determination of Phosphate

The method is described in the (b) (4) All validation criteria were met.

3.2.P.5.2.4.3 Determination of Citrate

The method is described in the (b) (4)

Reviewer's Comment
The method was not validated sufficiently. Accuracy and Linearity should be validated within the method range. A DBSQC reviewer had similar concern and sent an IR to resolve the issue. By the time of the review completion, the issue has not been resolved.

3.2.P.5.2.4.4 Determination of Sucrose

The method is described in the (b) (4)

Reviewer's Comment
The method was not validated sufficiently. Range should be validated using the DP samples, instead of the standard solution. Also, Accuracy and Linearity should be re-validated within this range. A DBSQC reviewer had similar concern and sent an IR to resolve the issue. By the time of the review completion, the issue has not been resolved.

3.2.P.5.2.4.5 Determination of Sodium

The method is described in the (b) (4)

All acceptance criteria were met.

Reviewer's Comment
Linearity needs to be re-validated using not only standards but the DP. Also, Parallelism between the standard and spiked samples was not demonstrated. A DBSQC reviewer had similar concern and sent an IR to resolve the issue. By the time of the review completion, the issue has not been resolved.

3.2.P.5.2.5 Impurities

3.2.P.5.2.5.1 Determination of Factor II

Human coagulation factor II (FII, prothrombin) is assayed (b) (4)

Reviewer's Comment
Accuracy, Linearity, Quantification Limit and Robustness should be validated using the DP matrix. Specificity should be also validated. A DBSQC reviewer had similar concern and sent an IR to resolve the issue. By the time of the review completion, the issue has not been resolved.

3.2.P.5.2.5.2 Determination of Factor IX

The test follows the curren (b) (4) method for the assay of human coagulation Factor IX (FIX). FIX activity is determined by (b) (4)

Reviewer's Comment

Accuracy, Quantitation Limit, Linearity, Range and Robustness should be validated using the DP matrix. A DBSQC reviewer had similar concern and sent an IR to resolve the issue. By the time of the review completion, the issue has not been resolved.

3.2.P.5.2.5.3 Determination of (b) (4)

(b) (4)

[redacted]

3.2.P.5.2.5.4 Determination of (b) (4)

(b) (4)

[redacted]

3.2.P.5.2.5.5 Determination of (b) (4)

(b) (4)

3.2.P.6 Reference Materials

The following reference standards and materials are used in the routine testing of the DP.

3.2.P.6.1 Factor X Standard

FX activity in DP is measured against a FX standard preparation. This is either the WHO IS for Factors II and X Concentrate, (currently the 3rd IS) for Factors II and X Concentrate, (b) (4) or in-house standard calibrated against the IS.

3.2.P.6.2 Protein Standard

Protein concentration in the DP is measured against a protein standard prepared from (b) (4)

3.2.P.6.3 Non-activated Partial Thromboplastin Time Control

The non-activated partial thromboplastin time (NAPTT) measures the absolute clotting time of a treated sample. There is no standard for this test. However, each test includes a positive control sample prepared from a prothrombin complex concentrate process intermediate to demonstrate NAPTT within a range close to the test limit ((b) (4)).

3.2.P.6.4 Fibrinogen Clotting Time Control

The fibrinogen clotting time (FCT) measures the absolute clotting time of a sample. There is no standard for this test. However, each test includes a(b) (4)

3.2.P.6.5 Factor II Standard

FII activity in the DP is measured against a FII standard preparation. This is either the WHO IS for FII and FX Concentrate, (currently the 3rd I S for FII and FX Concentrate, (b) (4) or in-house standard calibrated against the IS.

3.2.P.6.6 Factor IX Standard

FIX activity in the DP is measured against a FIX standard preparation. This is either the (b) (4) or in-house standard calibrated against the IS.

Communication with the Applicant for Additional Information

For the majority of concerns, DBSQC sent IRs on October 2, 2013, October 9, 2013 and September 4, 2013 to the Applicant. These requests were to validate methods, which were not validated (Determination of Non-activated Partial Thromboplastin Time (NaPTT) and Determination of Fibrinogen Clotting Time), and to re-validate relevant parameters of other methods that were found to have deficiencies (as reviewed above). In Amendments 14 (October 21, 2013) and 16 (November 15, 2013), the Applicant provided some information and indicated that they would complete these (validation and re-validation studies) by January 31, 2014. The responses were reviewed by Dr. Lokesh Bhattacharyya (DBSQC) who found that the concerns had not been addressed, as reflected in his memo.

For concerns that were additional to those managed by DBSQC, an IR was sent to the Applicant on November 22, 2013 (questions 8-11), and the Applicant responded on December 5, 2013 (Amendment 19) and on January 31, 2014 (Amendment 26) as follows.

Question 1
In validation of the Determination of Moisture by the (b) (4) Method, you expressed the moisture parameter in (b) (4) which is unclear. At the same time, in the drug product specification, this parameter is defined as (b) (4) Please express your validation results in the same units.

Response
On Jan 31, the Applicant provided data using the correct units, (b) (4) (Appendix 1).

Reviwer Comment
Response is acceptable

Question 2
In validation of the Determination of (b) (4)

Response
(b) (4)

(b) (4)

Question 3
For all validation studies that have been performed and are being planned during the review process, please submit study reports, in addition to study summaries.

Response
Validation Study reports will be provided for all validation work carried out by January 31, 2014. For the studies, submitted on January 31, 2014, the respective reports were provided.

Reviewer Comment
Responses to issues 1 through 3 above are acceptable. At the same time, by the review due day, February 21, 2014, the major issues communicated via the DBSQC reviewer have not yet been resolved; the latest amendment (31) was received on February 14, 2014.

REVIEWER'S COMMENTS

By the review due date (February 21, 2014), the information demonstrating validation of a number of assays has not yet been provided. Dr. Lokesh Bhattacharyya (DBSQC) has concerns about these assays as well. The non-validated methods are the following.

1. Determination of Factor X (Chromogenic Assay) (potency)
2. (b) (4) Moisture Determination
3. Determination of Total Protein by (b) (4)
4. Determination of Non-Activated Partial Thromboplastin Time (NaPTT) (Clotting Assay)
5. Determination of Fibrinogen Clotting Time (FCT) at (b) (4)
6. Determination of Factor II ((b) (4) Assay)
7. Determination of Factor IX ((b) (4) Assay)
8. Determination of (b) (4)
9. Determination of Citrate
10. Determination of (b) (4)
11. Sucrose Determination by (b) (4)
12. Determination of Sodium Content by (b) (4)
13. Determination of (b) (4)

I am not providing relevant comments for the Applicant, as in his review memo, Dr. Lokesh has already done this in a letter-ready form.

RECOMMENDATION

At this time, approval is not recommended. The Applicant needs to resolve the found deficiencies in the analytical methods validation.